# abcam

# Product datasheet

# Anti-GFAP antibody [EPR1034Y] ab68428



重组 RabMAb

★★★★★ 15 Abreviews 74 References 29 图像

#### 概述

产品名称 Anti-GFAP抗体[EPR1034Y]

描述 兔单克隆抗体[EPR1034Y] to GFAP - N-terminal

宿主 Rabbit

经测试应用 适用于: WB, IP, IHC-P, ICC/IF, mIHC, Flow Cyt (Intra), IHC-Fr

种属反应性 与反应: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

阳性对照 mIHC: Human cerebrum tissue and human cerebellum tissue.

常规说明 This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity - Long-term security of supply - Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

### 性能

形式

存放说明 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

存储溶液 pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.21% BSA

纯度 Protein A purified

克隆 单克隆 克隆编号 **EPR1034Y** 

同种型 lqG

## The Abpromise guarantee Abpromise™承诺保证使用ab68428于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		1/10000. Detects a band of approximately 50 kDa (predicted molecular weight: 50 kDa).  For unpurified use at 1/50 000 - 1/100 000.
IP		1/20 - 1/40.
IHC-P	****(9)	1/250 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.  See IHC antigen retrieval protocols.
ICC/IF	<b>★★★★</b> ☆ <u>(1)</u>	Use a concentration of 0.1 - 1 µg/ml.
mIHC		Use at an assay dependent concentration.
Flow Cyt (Intra)		1/500.
IHC-Fr	<b>★★★★ (3)</b>	1/250.

#### 靶标

功能 GFAP, a class-Ill intermediate filament, is a cell-specific marker that, during the development of

the central nervous system, distinguishes astrocytes from other glial cells.

组织**特异性** Expressed in cells lacking fibronectin.

疾病相关 Defects in GFAP are a cause of Alexander disease (ALEXD) [MIM:203450]. Alexander disease

is a rare disorder of the central nervous system. It is a progressive leukoencephalopathy whose hallmark is the widespread accumulation of Rosenthal fibers which are cytoplasmic inclusions in astrocytes. The most common form affects infants and young children, and is characterized by progressive failure of central myelination, usually leading to death usually within the first decade. Infants with Alexander disease develop a leukoencephalopathy with macrocephaly, seizures, and psychomotor retardation. Patients with juvenile or adult forms typically experience ataxia, bulbar

signs and spasticity, and a more slowly progressive course.

序列相似性 Belongs to the intermediate filament family.

翻译后修饰 Phosphorylated by PKN1.

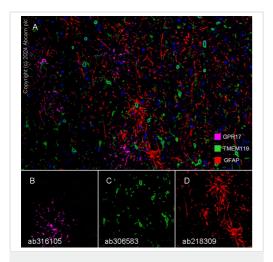
细胞定位 Cytoplasm. Associated with intermediate filaments.

图片



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFAP antibody
[EPR1034Y] (ab68428)

Immunohistochemical analysis of formalin fixed paraffin embedded human cerebellum labelling GFAP with ab68428 at a dilution of 1/1000. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an ChromoMap DAB (RUO) IHC Detection Kit with anti rabbit HQ and anti HQ HRP. Heat mediated antigen retrieval was conducted for 24 min with DISCOVERY cell conditioning solution (CC1) 100°C, pH 8.5. ab68428 was incubated at 37°C for 16 min. Sections were counterstained is with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.



Multiplex immunohistochemistry - Anti-GFAP antibody [EPR1034Y] (ab68428)

Fluorescence multiplex immunohistochemical analysis of human cerebrum tissue (Formalin/PFA-fixed paraffin-embedded sections).

Panel A: merged staining of anti-GPR17 (magenta; Opal™690), anti-TMEM119 (green; Opal™520) and anti-GFAP (red; Opal™570) on human cerebrum.

Panel B: anti-GPR17 staining oligodendrocytes in human cerebrum.

Panel C: anti-TMEM119 staining microglia in human cerebrum.

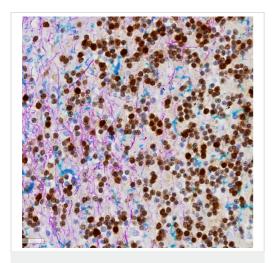
Panel D: anti-GFAP staining astrocytes in human cerebrum.

The section was incubated in three rounds of staining: in the order of <a href="mailto:ab316105">ab316105</a> at 1/500 dilution, <a href="mailto:ab306583">ab306583</a> at 1:2000 dilution, and <a href="mailto:ab218309">ab218309</a> at 1:1000 dilution for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. The secondary was Opal Polymer HRP Ms + Rb and counterstaining with DAPI.

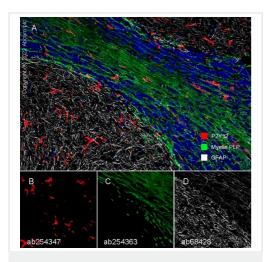
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

The immunostaining was performed on a Leica Biosystems
BOND® RX instrument with an Opal™ 4-color kit. Image acquisition
was performed with Leica SP8 confocal microscope.

This data was developed using <u>ab218309</u>, the same antibody clone in a different buffer formulation.



Multiplex immunohistochemistry - Anti-GFAP antibody [EPR1034Y] (ab68428)

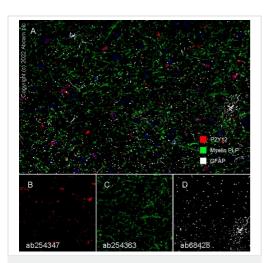


Multiplex immunohistochemistry - Anti-GFAP antibody [EPR1034Y] (ab68428)

Chromogenic multiplex immunohistochemical staining of FFPE normal human cerebellum tissue. **ab177487**, anti-NeuN DAB chromogen. Ab68428, anti-GFAP purple chromogen and **ab178846**, anti- lba1 teal chromogen plus haematoxylin counterstain.

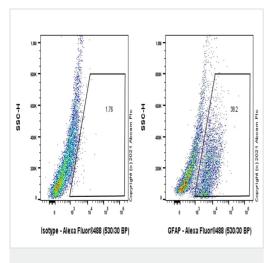
Chromogenic immunostaining was performed on a Roche Ventana Discovery Ultra instrument. The section was deparaffinised and incubated with CC1 solution for 24min 100°C. Following this with 3 rounds of staining in the order of <a href="mailto:ab177487">ab177487</a> (1/600), <a href="mailto:ab178846">ab178846</a> (1/4000) ab68428 (1/1000). Between rounds of staining, antibody denaturation was conducted using Ultra CC2 solution for 8min at 100°C to avoid cross reactivity. Signal was developed with antirabbit HQ followed by anti-HQ HRP coupled with Chromomap DAB kit, Discovery purple or Discovery teal chromogens and haematoxylin II counterstain.

Fluorescence multiplex immunohistochemical analysis of the human cerebellum (Formalin/PFA-fixed paraffin-embedded sections). Panel A: merged staining of anti-GFAP (ab68428, gray; Opal™690), anti-Myelin PLP (<u>ab254363</u>, green; Opal™520) and anti-P2Y12 (ab254347 red; Opal™570) on human cerebellum. Panel B: anti-P2Y12 stained on microglial cells. Panel C: anti-Myelin PLP stained on myelin sheaths of oligodendrocytes. Panel D: anti-GFAP stained on astrocytes. Opal Polymer HRP Ms + Rb was used as a secondary antibody. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. The section was incubated in three rounds of staining: in the order of ab68428 (1/50 dilution), ab254363 (1/2000 dilution), and ab254347 (1/1000 dilution) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-GFAP antibody [EPR1034Y] (ab68428)

Fluorescence multiplex immunohistochemical analysis of the human cerebrum (Formalin/PFA-fixed paraffin-embedded sections). Panel A: merged staining of anti-GFAP (ab68428, gray; Opal™690), anti-Myelin PLP (ab254363, green; Opal™520) and anti-P2Y12 (ab254347, red; Opal™570) on human cerebrum. Panel B: anti-P2Y12 stained on microglial cells. Panel C: anti-Myelin PLP stained on myelin sheaths of oligodendrocytes. Panel D: anti-GFAP stained on astrocytes. Opal Polymer HRP Ms + Rb was used as a secondary antibody. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument with an Opal<sup>™</sup> 4-color kit. The section was incubated in three rounds of staining: in the order of ab68428 (1/50 dilution), ab254363 (1/2000 dilution), and ab254347 (1/1000 dilution) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Leica SP8 confocal microscope.

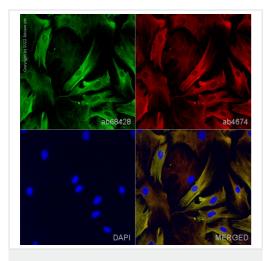


Flow Cytometry (Intracellular) - Anti-GFAP antibody [EPR1034Y] (ab68428)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Mouse primary brain cells cells labelling GFAP with ab68428 at 1/500 dilution (0.1ug)/ Right compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFAP antibody
[EPR1034Y] (ab68428)



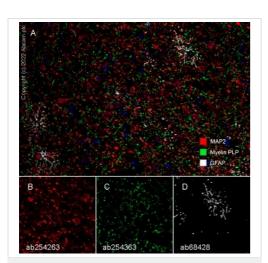
Immunocytochemistry/ Immunofluorescence - Anti-GFAP antibody [EPR1034Y] (ab68428)

IHC image of GFAP staining in a formalin fixed, paraffin embedded normal human hippocampus tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pretreated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab68428 at 1/100 dilution for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

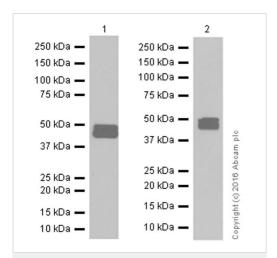
Immunofluorescence staining of GFAP using ab68428 in primary rat hippocampal mixed glia, (prepared from P2 rat hippocampal brain area, obtained from Transnetyx Tissue by BrainBits, LLC, cat.no. SDPHP4m), DIV4. The cells were fixed with 100% MeOH (5 min), permeabilized with 0.1% Triton-X-100 (in PBS) for 5 mins and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab68428 at 0.1 µg/ml and ab4674, Anti-GFAP antibody, at 1/1000 dilution. Cells were then incubated with ab150081, Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and ab150176, Goat Anti-Chicken lgY H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Images were acquired with the Perkin Elmer Operetta HCA and a maximum intensity projection of confocal sections is shown. The antibody ab68428 gave comparable results using 4% formaldehyde fixation (10 min).



Multiplex immunohistochemistry - Anti-GFAP antibody [EPR1034Y] (ab68428)

Fluorescence multiplex immunohistochemical analysis of human cerebrum tissue (formalin/PFA-fixed paraffin-embedded section). Panel A: merged staining of anti-GFAP (ab68428, gray; Opal™690), anti-Myelin PLP (ab254363, green; Opal™520) and anti-MAP2 (ab254263, red; Opal™570) on human cerebrum tissue. Panel B: anti-MAP2 stained cell body and dendrites of neurons. Panel C: anti-Myelin PLP stained on myelin sheaths of oligodendrocytes. Panel D: anti-GFAP stained on astrocytes. Opal Polymer HRP Ms + Rb was used as a secondary antibody. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. The section was incubated in three rounds of staining: in the order of ab68428 (1/50 dilution), ab254363 (1/2000 dilution), and ab254263 (1/4000 dilution) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) was used for 20 mins. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Leica SP8 confocal microscope.



Western blot - Anti-GFAP antibody [EPR1034Y] (ab68428)

**All lanes :** Anti-GFAP antibody [EPR1034Y] (ab68428) at 1/10000 dilution

Lane 1: Human cerebellum tissue lysate at 20 µg

Lane 2 : Human brain tissue lysate at 10 µg

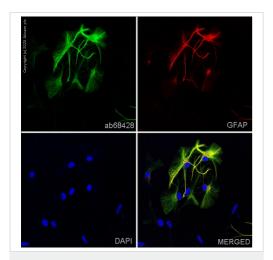
#### Secondary

**All lanes :** Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/2000 dilution

Predicted band size: 50 kDa

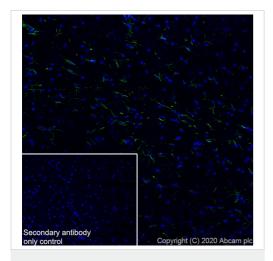
Observed band size: 48-50 kDa

Blocking and Diluting buffer 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-GFAP antibody [EPR1034Y] (ab68428)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized mouse primary neural mix culture cells labelling GFAP with ab68428 at 1/250 dilution, followed by **ab150077** AlexaFluor<sup>®</sup>488 Goat anti-Rabbit secondary antibody at 1/1000 dilution (Green). Confocal scanning Z step was set as 0.3 µm followed by image processing with maximum Z projection. **ab10062** anti-GFAP antibody [GF5] at 1/100 dilution followed by **ab150120** Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) at 1/1000 (2µg/ml) was used as a counterstain. The Nuclear counterstain was DAPI (Blue).

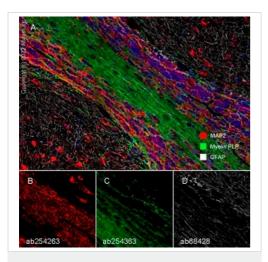


Immunohistochemistry (Frozen sections) - Anti-GFAP antibody [EPR1034Y] (ab68428)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Rat cerebrum tissue labeling GFAP with ab68428 at 1/1000 dilution (2.011  $\mu$ g/mL) followed by <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) at 1/1000 dilution (2  $\mu$ g/mL) (Green). Positive staining on rat cerebrum is observed. The nuclear counterstain was DAPI (Blue).

Secondary antibody control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) at 1/1000 dilution (2 µg/mL).<\p>

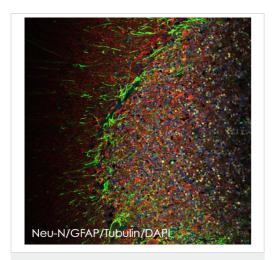
Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).



Multiplex immunohistochemistry - Anti-GFAP antibody [EPR1034Y] (ab68428)

Fluorescence multiplex immunohistochemical analysis of human cerebellum tissue (formalin/PFA-fixed paraffin-embedded section). Panel A: merged staining of anti-GFAP (ab68428, gray; Opal™690), anti-Myelin PLP (ab254363, green; Opal™520) and anti-MAP2 (ab254263, red; Opal™570) on human cerebellum tissue. Panel B: anti-MAP2 stained cell body and dendrites of neurons. Panel C: anti-Myelin PLP stained on myelin sheaths of oligodendrocytes. Panel D: anti-GFAP stained on astrocytes. Opal Polymer HRP Ms + Rb was used as a secondary antibody. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. The section was incubated in three rounds of staining: in the order of ab68428 (1/50 dilution), ab254363 (1/2000 dilution), and ab254263 (1/4000 dilution) for 30 mins at room temperature. Each round was followed by a separate

fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) was used for 20 mins. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-GFAP antibody [EPR1034Y] (ab68428)

Fluorescence multiplex immunohistochemical analysis of human cerebellum tissue (formalin-fixed paraffin-embedded section).

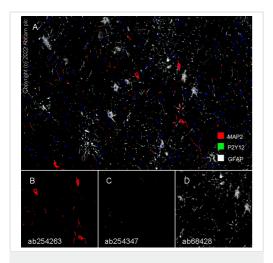
Merged staining of Neu-N (<u>ab177487</u>; yellow; Opal<sup>™</sup>570), antibeta III Tubulin (<u>ab52623</u>; red; Opal<sup>™</sup>690) and anti-GFAP (ab68428; green; Opal<sup>™</sup>520).

The immunostaining was performed on a Leica Biosystems  $BOND^{\circledR}RX$  instrument with an  $Opal^{\intercal M}$  kit.

The section was incubated in three rounds of staining with <a href="mailto:ab177487"><u>ab177487</u></a> (1/1000 dilution), <a href="mailto:ab52623"><u>ab52623</u></a> (1/200 dilution) and <a href="mailto:ab68428"><u>ab68428</u></a> (1/250 dilution); each using a separate fluorescent tyramide signal amplification system.

Sodium citrate antigen retrieval (pH 6.0) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity.

DAPI (blue) was used as a nuclear counter stain.



Multiplex immunohistochemistry - Anti-GFAP antibody [EPR1034Y] (ab68428)

Fluorescence multiplex immunohistochemical analysis of the Human cerebrum (Formalin/PFA-fixed paraffin-embedded sections).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

Opal Polymer HRP Ms + Rb was used as a secondary antibody.DAPI (blue) was used as a nuclear counter stain.

Panel A: Merged staining of anti-GFAP (gray; Opal<sup>™</sup>690), anti-P2Y12 (green; Opal<sup>™</sup>520) and anti-MAP2 (red; Opal<sup>™</sup>570) on human cerebrum.

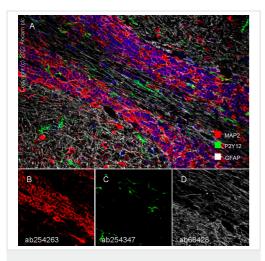
Panel B: Anti-MAP2 stained cell body and dendrites of neurons.

Panel C: Anti-P2Y12 stained on microglial cells.

Panel D: Anti-GFAP stained on astrocytes.

The section was incubated in three rounds of staining: in the order of ab68428, <u>ab254347</u>, and <u>ab254263</u> for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems
BOND® RX instrument with an Opal™ 4-color kit. Image acquisition
was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-GFAP antibody [EPR1034Y] (ab68428)

Fluorescence multiplex immunohistochemical analysis of the Human cerebellum (Formalin/PFA-fixed paraffin-embedded sections).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

Opal Polymer HRP Ms + Rb was used as a secondary antibody.DAPI (blue) was used as a nuclear counter stain.

Panel A: Merged staining of anti-GFAP (gray; Opal™690), anti-P2Y12 (green; Opal™520) and anti-MAP2 (red; Opal™570) on human cerebellum.

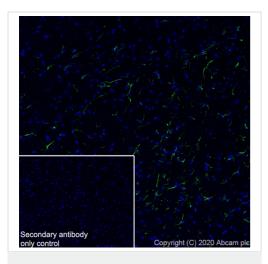
Panel B: Anti-MAP2 stained cell body and dendrites of neurons.

Panel C: Anti-P2Y12 stained on microglial cells.

Panel D: Anti-GFAP stained on astrocytes.

The section was incubated in three rounds of staining: in the order of ab68428, <u>ab254347</u>, and <u>ab254263</u> for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems
BOND® RX instrument with an Opal™ 4-color kit. Image acquisition
was performed with Leica SP8 confocal microscope.

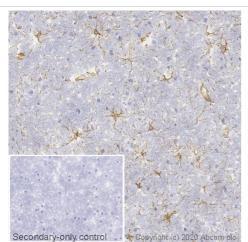


Immunohistochemistry (Frozen sections) - Anti-GFAP antibody [EPR1034Y] (ab68428)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Mouse cerebrum tissue labeling GFAP with ab68428 at 1/1000 dilution (2.011  $\mu$ g/mL) followed by <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) at 1/1000 dilution (2  $\mu$ g/mL) (Green). Positive staining on mouse cerebrum is observed. The nuclear counterstain was DAPI (Blue).

Secondary antibody control: Secondary antibody is **ab150077** Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) at 1/1000 dilution (2 µg/mL).<\p>

Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).



Immunohistochemistry (Frozen sections) - Anti-GFAP antibody [EPR1034Y] (ab68428)

All lanes: Anti-GFAP antibody [EPR1034Y] (ab68428) at 1/10000 dilution

For other IHC staining systems (automated and non-automated)

customers should optimize variable parameters such as antigen

retrieval conditions, primary antibody concentration and antibody

Lane 1: Mouse cerebellum tissue lysate

IHC image of GFAP staining in a section of frozen

normal human cerebral cortex performed on a Leica BOND<sup>TM</sup> system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab68428, 1/250 dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical

Lane 2: Mouse brain tissue lysate

assay without primary antibody.

incubation times.

Lysates/proteins at 20 µg per lane.

250 kDa -150 kDa -100 kDa -75 kDa 🕳 50 kDa -Copyright (c) 2016 Abcam plo 37 kDa 🕳 25 kDa 🕳 20 kDa -15 kDa -10 kDa 🕳

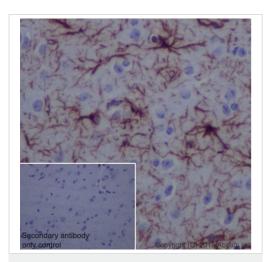
Western blot - Anti-GFAP antibody [EPR1034Y] (ab68428)

#### Secondary

All lanes: Anti-Rabbit IgG (HRP), specific to the non-reduced form of IqG at 1/2000 dilution

Predicted band size: 50 kDa Observed band size: 50 kDa

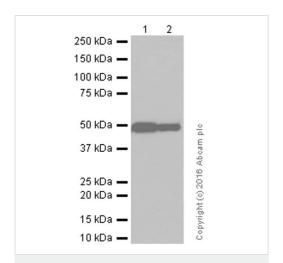
Blocking and Diluting buffer 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFAP antibody

[EPR1034Y] (ab68428)

Immunohistochemical analysis of paraffin-embedded mouse cerebral cortex tissue sections labelling GFAP with purified ab68428 at a dilution of 1/500. The secondary antibody used was **ab97051**, Goat Anti-Rabbit IgG H&L (HRP) at a dilution of 1/500. The sample was counterstained with hematoxylin. Antigen retrieval was performed using EDTA Buffer; pH 9.0. PBS was used instead of the primary antibody as the negative control and is shown in the inset.



Western blot - Anti-GFAP antibody [EPR1034Y] (ab68428)

**All lanes :** Anti-GFAP antibody [EPR1034Y] (ab68428) at 1/50000 dilution

Lane 1: Rat cerebellum tissue lysate

Lane 2: Rat brain tissue lysate

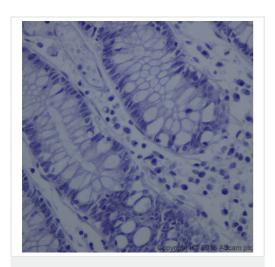
Lysates/proteins at 20 µg per lane.

#### **Secondary**

**All lanes :** Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG at 1/2000 dilution

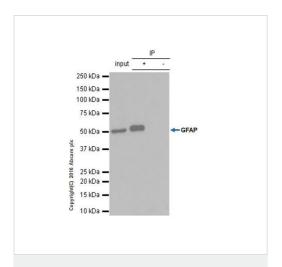
**Predicted band size:** 50 kDa **Observed band size:** 50 kDa

Blocking and Diluting buffer 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFAP antibody
[EPR1034Y] (ab68428)

Immunohistochemical analysis of paraffin-embedded human colon tissue sections labelling GFAP with purified ab68428 at a dilution of 1/500. The secondary antibody used was **ab97051**, Goat Anti-Rabbit lgG H&L (HRP) at a dilution of 1/500. The sample was counterstained with hematoxylin. Antigen retrieval was performed using EDTA Buffer; pH 9.0.



Immunoprecipitation - Anti-GFAP antibody [EPR1034Y] (ab68428)

ab68428 at 1/20 dilution immunoprecipitating GFAP in rat brain whole cell lysate observed at 50 KDa (lanes 1 and 2).

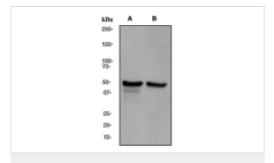
Lane 1 (input): Rat brain whole cell lysate 10ug

Lane 2 (+): ab68428 + Rat brain whole cell lysate

Lane 3 (-): Rabbit monoclonal  $\log G$  (ab172730) instead of ab68428 in Rat brain whole cell lysate

For western blotting, ab68428 was used followed by VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10,000 dilution.

Blocking and Diluting buffer and concentration: 5% NFDM/TBST.



Western blot - Anti-GFAP antibody [EPR1034Y] (ab68428)

**All lanes**: Anti-GFAP antibody [EPR1034Y] (ab68428) at 1/5000 dilution (unpurified)

Lane 1: Human brain lysate

Lane 2: Rat brain lysate

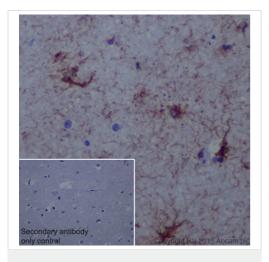
Lysates/proteins at 10 µg per lane.

#### **Secondary**

All lanes: HRP labelled Goat anti-Rabbit antibody at 1/2000

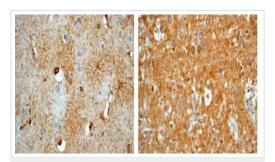
dilution

Predicted band size: 50 kDa
Observed band size: 50 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFAP antibody
[EPR1034Y] (ab68428)

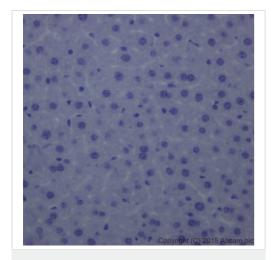
Immunohistochemical analysis of paraffin-embedded human cerebral cortex tissue sections labelling GFAP with purified ab68428 at a dilution of 1/500. The secondary antibody used was **ab97051**, Goat Anti-Rabbit IgG H&L (HRP) at a dilution of 1/500. The sample was counterstained with hematoxylin. Antigen retrieval was performed using EDTA Buffer; pH 9.0. PBS was used instead of the primary antibody as the negative control and is shown in the inset.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFAP antibody
[EPR1034Y] (ab68428)

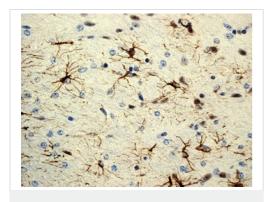
Immunohistochemical analysis of formalin-fixed paraffin-embedded human brain (left) and human glioma (right) tissue sections labelling GFAP with unpurified ab68428 at dilution of 1/250.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFAP antibody [EPR1034Y] (ab68428)

Immunohistochemical analysis of paraffin-embedded mouse liver tissue sections labelling GFAP with purified ab68428 at a dilution of 1/500. The secondary antibody used was ab97051, Goat Anti-Rabbit IgG H&L (HRP) at a dilution of 1/500. The sample was counterstained with hematoxylin. Antigen retrieval was performed using EDTA Buffer; pH 9.0.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFAP antibody [EPR1034Y] (ab68428)

Immunohistochemical analysis of formalin-fixed paraffin-embedded mouse brain tissue section labelling GFAP with unpurified ab68428 at dilution of 1/250.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.





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Anti-GFAP antibody [EPR1034Y] (ab68428)

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